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Research report

Altered neural activity in the '*when*' pathway during temporal processing in fragile X premutation carriers

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HIGHLIGHTS

- Neurotypical adults show greater right TPJ in temporal than in spatial processing.
- The increased right TPJ activation was not found in fragile X premutation carriers.
- Elevated *FMR1* mRNA explained atypical right TPJ activity in the premutation group.

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ABSTRACT

Mutations of the fragile X mental retardation 1 (*FMR1*) gene are the genetic cause of fragile X syndrome (FXS). Large expansions of the CGG repeat (>200 repeats) consequently result in transcriptional silencing of the *FMR1* gene and deficiency/absence of the *FMR1* protein (FMRP). Carriers with a premutation allele (55–200 of CGG repeats) are often associated with mildly reduced levels of FMRP and/or elevated levels of *FMR1* mRNA. Recent studies have shown that infants with FXS exhibit severely reduced resolution of temporal attention, whereas spatial resolution of attention is not impaired. Following from these findings in the full mutation, the current study used fMRI to examine whether premutation carriers would exhibit atypical temporal processing at behavioral and/or neural levels. Using spatial and temporal working memory (SWM and TWM) tasks, separately tagging spatial and temporal processing, we demonstrated that neurotypical adults showed greater activation in the '*when* pathway' (i.e., the right temporoparietal junction: TPJ) during TWM retrieval than SWM retrieval. However, premutation carriers failed to show this increased involvement of the right TPJ during retrieval of temporal information. Further, multiple regression analyses on right TPJ activation and *FMR1* gene expression (i.e., CGG repeat size and *FMR1* mRNA) suggests that elevated *FMR1* mRNA level is a powerful predictor accounting for reduced right TPJ activation associated with temporal processing in premutation carriers. In conclusion, the current study provides the first evidence on altered neural correlates of temporal processing in adults with the premutation, explained by their *FMR1* gene expression.

1. Introduction

The ability to mentally represent and process temporal information accurately is an essential component of cognitive functioning in everyday life. For example, when executing a familiar sequence of events, such as assembling a sandwich or paying a bill, organizing and remembering the temporal order of the required actions is critical to complete the task accurately and efficiently. Even something as simple as remembering one's phone number, requires processing of temporal information. Despite its central role in everyday life, it is only recently that researchers have investigated the development of, and neural mechanisms involved in, temporal processing in humans.

With regards to the development of temporal processing, Farzin et al. (2011) recently reported reduced temporal resolution of visual attention in infants compared to that of adults [1]. Specifically, temporal frequency thresholds were measured in infants (6–15 months) using a flicker task. It was found that the temporal resolution of infants was significantly coarser (up to 1 Hz) than that of adults (up to 10 Hz) previously reported [2,3]. Further, atypical development of temporal processing in young children with fragile X syndrome (FXS) was also investigated [4].

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FXS is the most common form of inherited intellectual disability, which results from large expansions of the CGG trinucleotide repeat in the promoter region of the fragile X mental retardation 1 (*FMR1*) gene located at Xq27.3. When CGG repeat size exceeds 200, silencing of the gene occurs, resulting in reduction or absence of the gene's protein product (fragile X mental retardation 1 protein: FMRP). When CGG repeat sizes range between 55 and 200 (1 of 130–250 females and 1 of 250–800 males; [5]), the gene is unmethylated, but these so-called "premutation carriers" are often associated with mildly reduced levels of FMRP and/or elevated levels of *FMR1* mRNA [6–9]. Individuals with the fragile X premutation are also associated with the risk of developing a late-onset neurodegenerative disorder known as fragile X-associated tremor/ataxia syndrome (FXTAS) [10,11]. Using crowding and flicker tasks, which measured resolution of spatial and temporal visual attention, respectively, Farzin and colleagues (2011) demonstrated that infants with FXS showed greatly reduced resolution of temporal attention (up to 0.5 Hz) compared to their typically developing counterparts. By contrast, the resolution of their spatial attention was not different. Further, the researchers found that the coarse resolution of temporal attention was genetically dosage sensitive such that greater CGG repeat length was associated with lower temporal resolution in infants with FXS. Such findings of impaired temporal processing in infants with FXS have implications on their later development of visual functions that require precise temporal sensitivity, such as motion perception, visual tracking, and memory for temporal order.

The above-mentioned findings of impaired temporal processing in infants with FXS is also in line with recent findings from animal studies using CGG knock-in (KI) mice modeling the FX premutation. Specifically, using a temporal ordering of spatial locations task, Borthwell and colleagues (2012) have found that female CGG KI mice showed reduced spatial and temporal resolution, and the impairment was *FMR1* genetic dosage sensitive indexed by the CGG repeat size [12]. Similarly, Hunsaker et al. (2010) also demonstrated that female KI mice with higher CGG repeat expansions (i.e., 150–200 repeats) performed more poorly on a task for temporal order memory than wildtype mice, whereas those with lower repeat expansions (80–100 CGG) performed similarly to wildtype mice [13]. Further, using tasks requiring spatial and temporal pattern separation, the researchers demonstrated that CGG repeat length negatively modulated spatiotemporal attention in male CGG KI mice [14].

Despite evidence of impaired spatiotemporal processing in CGG KI mice which models possible impairments in spatial and/or temporal functions in individuals with the FX premutation, there are currently no studies that have examined the extent of impairments in temporal processing in carriers with the fragile X premutation either at the behavioral or the neural level. Instead, recent studies have reported impairments in visuospatial processing in adults with the FX premutation without FXTAS (i.e., asymptomatic adults with the FX premutation) [15–18]. In particular, Keri and Benedek [15,16] reported that adults with the FX premutation revealed subtle impairments on tests requiring magnocellular visual pathways projecting to cortical areas responsible for motion perception and visuospatial processing (dorsal occipito-parietal stream). Specifically, adults with the premutation performed a visual contrast sensitivity task consisting of two types of vertical sinusoidal luminance contrast gratings. The two types of gratings had different spatiotemporal properties to bias information processing toward the M (magnocellular) and P (parvocellular) pathways (M pathway: low spatial/high temporal frequency; P pathway: high spatial/low temporal frequency). Using such stimuli, the authors found that premutation carriers had lower contrast sensitivity than controls in the M pathway condition, but not in the P pathway condition, indicating subtle impairments in motion and visouspatial processing in premutation carriers. Furthermore, Hocking and colleagues [17] demonstrated that asymptomatic male premutation carriers with high CGG repeat sizes ($100 < CGG < 200$) performed significantly worse than normal controls on a Dot test of visuospatial working memory (WM). However, notwithstanding evidence of atypical temporal resolution in infants with FXS and findings in CGG KI mice of impairments in temporal order memory, impairments in temporal processing in individuals with the FX premutation remain unspecified.

Thus, in the current study, we investigated whether temporal processing is impaired in asymptomatic adults with the FX premutation at behavioral and/or neural levels. Specifically, we investigated neural correlates associated with temporal processing in neurotypical adults, and examined whether adults with the FX premutation show atypicality in recruiting those regions to process temporal information. Recently, lesion and transcranial magnetic stimulation (TMS) studies provide evidence that the right parietal lobe, especially the right temporoparietal junction (TPJ), plays a core role to process temporal information required in temporal order judgment (*when* pathway; [19–22]). Hence, in the present study, we examined whether activation in the right TPJ associated with temporal processing is significantly reduced in asymptomatic adults with the FX premutation compared to their neurotypical counterparts.

To identify neural regions specifically involved in temporal processing, we developed two WM tasks, namely a spatial WM (SWM) and a temporal WM (TWM) task. The two tasks have identical stimuli and procedures, but the task requirement for each task was different: the SWM task required memory for spatial location, whereas the TWM task required memory for temporal order. Using fMRI and the two WM tasks, we tested the following hypotheses in the current study. First, we hypothesized that neurotypical adults without the premutation allele (i.e., controls) would activate the *when* pathway, namely the right TPJ, to a greater extent during retrieval of temporal order information than spatial location (i.e., in the contrast of TWM retrieval > SWM retrieval). Further, we hypothesized that FX premutation carriers would show attenuated activity in the right TPJ than the controls in the same contrast of interest. Finally, we expected to find a dosage response of *FMR1* gene expression in the FX premutation group, indexed by CGG repeat expansion and/or *FMR1* mRNA level, such that right TPJ activation associated with temporal processing would be significantly accounted for by the degree to which the *FMR1* gene is behaving normally.

2. Methods

2.1. Participants

Twenty asymptomatic young adults with the premutation (premutation group; mean age: 30.4 years, SD = 7.32; Female: 10) and 20 neurotypical, age-matched controls (control group; mean age: 29.7 years, SD = 6.07; Female: 10) with normal or corrected-to-normal vision participated in the current study. Allele status was confirmed by *FMR1* DNA testing. Full-scale IQ (FSIQ) was obtained using the Wechsler Adult Intelligence Scale, third edition (WAIS-III; [23]). Two male participants in the premutation group and 2 female participants in the control group were excluded from further analyses due to excessive movement (>3 mm in x, y, or z planes) during the task in the MRI scanner. As presented in Table 1, the two groups did not significantly differ in age ($t(34) = .11$, $p = .92$), FSIQ ($t(28) = 1.30$, $p = .20$), nor gender ($\chi^2 = .44$, $df = 1$, $p = .51$), but significantly differ in *FMR1* gene expressions: the premutation group showed increased CGG repeat size ($t(21.3) = 12.5$, $p < .001$) and elevated *FMR1* mRNA level ($t(18.9) = 7.5$, $p < .001$) compared to the control group. The two molecular variables showed a significant